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Flavonols of Licorice Root[1]

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The structure of a new flavonol licoflavonol (II), isolated from Seihoku Kanzo (*Glycyrrhiza* spp., Leguminosae) along with already known compounds kumatakenin (I), glycyrol (VI) and licoricone (VII), has been determined as 6-γ,γ-dimethylallyl-kaempferol by spectroscopic analysis.

Licorice is a name given to the roots of the genus *Glycyrrhiza* (Leguminosae) and has a sweet taste, due to the presence of glycyrrhizin. It has been used for a long time as a very important crude drug in China as well as in European countries incidentally. While the European licorice has been assigned to *Glycyrrhiza glabra*, it is said that Chinese licorice includes several species, namely *G. uralensis* Fischer et De Candolle, *G. glabra* L. var. *glandulifera* Regel et Herder and *G. echinata* L.. A member of Chinese licorice named Tohoku Kanzo (in Japanese) has been assigned to *G. uralensis*, whose constituents have been studied.[3]

Systematic investigation of various kinds of licorice has revealed the existence of a closely allied species of *G. uralensis*. While Tohoku Kanzo is produced in the northeast part of China, this licorice grows in the northwest part of the country and is named Seihoku Kanzo (in Japanese), but the species name is uncertain.

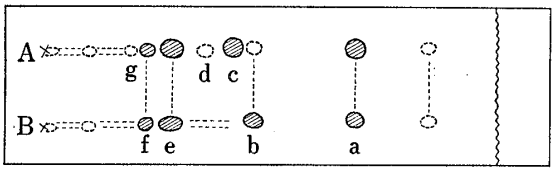

Fig. 1. TLC of the Methanol Extracts of Seihoku Kanzo (A) and Tohoku Kanzo (B)

a : licoricidin (=C-14)
b : isoglycyrol
c : kumatakenin (=C-11, I)
d : mixture of licoflavonol (=C-15, II), C-16 and C-17
e : glycyrol (=C-13, VI)
f : licoricone (=C-18, VII)
g : C-12
(benzene: ether=4:1, Silica gel GF_{254})

The methanolic extracts of the root bark of both species of licorice showed their close relationship on a thin-layer chromatogram as shown in Fig. 1. Among the major spots, however, the Seihoku Kanzo contains a peculiar spot, which was observed as a golden yellow fluorescent spot when exposed under a 365 nm ultraviolet lamp after being sprayed with H_2SO_4 and heated. So, a tentative name "C-11" was given to this compound which was isolated as a pure state by means of repeated column chromatography over silica gel and polyamide.

C-11 was obtained as yellow needles, mp 252—254°, and showed a positive magnesium-hydrochloric acid test. It gave an infrared (IR) absorption at 1662 cm^{-1} and showed ultraviolet (UV) absorption at 268.5 and 352.5 nm. These findings indicated that C-11 should be a flavonol. In the nuclear magnetic resonance (NMR) spectrum two methoxyls appeared at δ 3.80 and 3.85, and two hydroxyls at δ 10.20 and 12.56. The latter was assigned to a chelated hydroxyl, probably 5-OH. This was confirmed by a bathochromic UV shift by the addition of $AlCl_3$. NaOEt also induced a bathochromic shift though no shift occurred when NaOAc

1) Part XLI in the series of *Chemical Studies on the Oriental Plant Drugs*. Part XL: T. Kinoshita, T. Saitoh, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **24**, 991 (1976).
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3) *a*) S. Shibata and T. Saitoh, *Chem. Pharm. Bull.* (Tokyo), **16**, 1932 (1968); *b*) T. Saitoh and S. Shibata, *ibid.*, **17**, 729 (1969); *c*) M. Kaneda, T. Saitoh, Y. Iitaka, and S. Shibata, *ibid.*, **21**, 1338 (1973).

was added. These results suggested that C-11 has a methoxyl at 7-position and a hydroxyl at 4′-position.

Of six aromatic protons in the NMR spectrum of C-11, four composed A_2B_2 system at δ 6.90 and 7.92 with 8 Hz (ortho-coupling), while the other two protons were observed as AB type signals at δ 6.31 and 6.66 with 2 Hz (meta-coupling). All these data are in accord with structure I which has already been given to kumatakenin,[4] a constituent of *Alpinia kumatake* (Zingiberaceae). Actually, C-11 was proved to be identical with an authentic specimen of kumatakenin in all respect.

Extensive investigation led us to isolate another flavonol named licoflavonol as a minor constituent of the same plant source.

Licoflavonol (II), yellow needles, mp 185—187°, was formulated as $C_{20}H_{18}O_6$ in accordance with the elementary analysis. It showed positive reactions with $FeCl_3$ and Mg-HCl. The UV spectrum exhibited absorption maxima at 271 and 369 nm, closely analogous to that[5] of kaempferol. The NMR spectrum revealed the presence of a γ,γ-dimethylallyl group [δ 1.67 (3H, s), 1.77 (3H, s), 3.24 (2H, d, J=8), 5.18 (1H, t, J=8)], five aromatic protons and four hydroxyls which were also confirmed by the formation of a tetraacetate (III), mp 177—178°. As for aromatic protons, four protons composed A_2B_2 system (J=8 Hz) and one proton appeared as a singlet. This fact suggested that the γ,γ-dimethylallyl group is attached to

Table I. Comparison of Chemical Shifts of Aromatic Protons of Licoflavonol and Its Acetate with Related Compounds

Compounds	Aromatic protons				Solvents
	6	8	2′,6′	3′,5′	
Kaempferol	6.14	6.42	8.00	6.88	a
Licoflavonol (II)	—	6.45	7.98	6.89	a
Kaempferol tetraacetate	6.83	7.26	7.79	7.23	b
Licoflavonol tetraacetate (III)	—	7.25	7.79	7.20	b
Des-O-methyl-anhydroicaritin tetraacetate (V)	6.86	—	7.83	7.23	b

Figures are chemical shifts (δ) from TMS as the internal standard measured in d_6-DMSO (a) or in $CDCl_3$ (b).

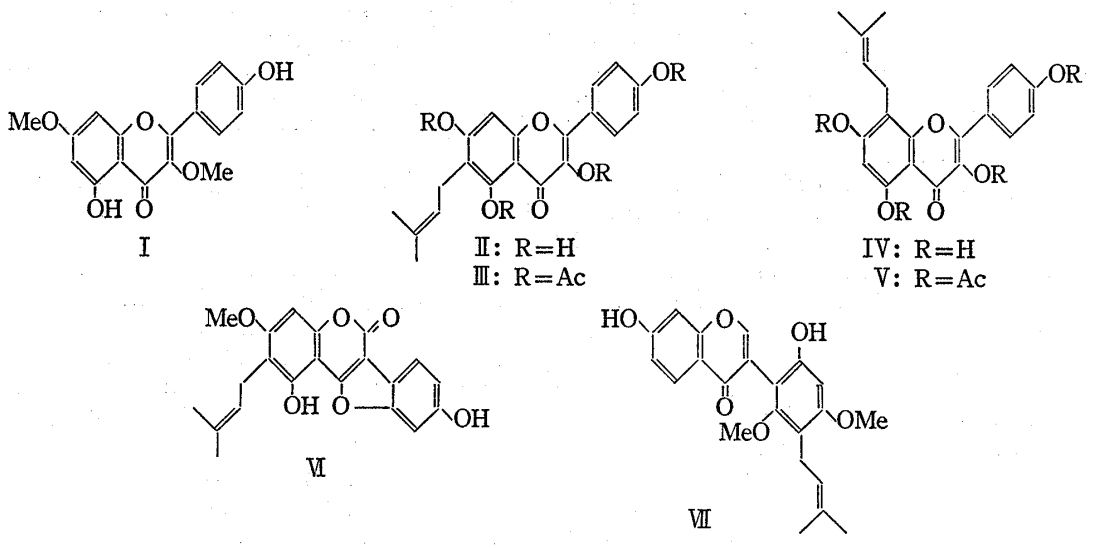

Chart 1

4) Y. Kimura, M. Takido, S. Takahashi, and M. Kimishima, *Yakugaku Zasshi*, **87**, 440 (1967).
5) T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, 1970.

A ring, not to B ring.

The above information leads to the conclusion that II is a flavonol, which can be represented as either II or IV. The latter, however, has been assigned to des-O-methyl-anhydroicaritin.[6] Confirmation of the structure (II) of licoflavonol was obtained by comparison of the chemical shifts of aromatic protons with those of the related compounds. As shown in Table I, a singlet signal appeared at δ 7.25 in the NMR spectrum of licoflavonol tetraacetate (III) should be assigned to H-8, not to H-6, because the chemical shift is in good agreement with that of H-8 proton of kaempferol tetraacetate.

This is the first example of isolation of flavonols from licorice roots though many flavonoid compounds have been isolated as the constituents of that drug.

By further investigation, C-13 and C-18 were obtained as major constituents. These were identified with a coumestan glycyrol (VI) and an isoflavone licoricone (VII), which were already isolated from Tohoku Kanzo.[3] The existence of glycyrol and licoricone in Seihoku Kanzo revealed that both plant species of Tohoku Kanzo and Seihoku Kanzo have botanically close relationship from the chemotaxonomical point of view.

Experimental

Dried and ground licorice roots (8 kg) were first percolated with *n*-hexane, and then extracted with chloroform at room temperature. Removal of the solvent gave the brown extract (205 g). The extract was chromatographed on a column of silica gel eluting with benzene containing increasing amounts of acetone (19: 1—4: 1) to give crude fractions A, B, C, D, E, and F. Further chromatography of each crude fraction on polyamide with methanol gave pure crystals, C-14 from A, C-11 from B, C-13 from D, C-18 from E, and C-12 from F. Elution of C with methanol on Sephadex LH 20 yielded C-15, C-16, and C-17 as pure crystals.

Kumatakenin (=C-11, I)——Kumatakenin was recrystallised from benzene–acetone to form yellow needles, mp 252—254°. It gave a red color with magnesium-hydrochloric acid. λ_{max}^{EtOH} nm (log ε): 268.5 (4.28), 302 sh (4.04), 352.5 (4.29). $\lambda_{max}^{EtOH+AcONa}$ nm: 268, 301 sh, 355. $\lambda_{max}^{EtOH+NaOEt}$ nm: 232, 260, 267 sh, 290 inf, 404. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 230 sh, 278, 304, 347, 400. ν_{max}^{KBr} cm^{-1}: 3240, 2940, 1662, 1601, 1585, 1497. $\delta_{ppm}^{d_6\text{-DMSO}}$: 3.80 (3H, s, OMe), 3.85 (3H, s, OMe), 6.31 (1H, d, 2, H–6), 6.66 (1H, d, 2, H–8), 6.90 (2H, d, 8, H–3′ and H–5′), 7.92 (2H, d, 8, H–2′ and H–6′), 10.20 (1H, s, OH–4′, exchangeable with D_2O), 12.56 (1H, s, OH–5, exchangeable with D_2O). Mass Spectrum *m/e*: 314 (M^+).

Licoflavonol (=C-15, II)——Licoflavonol was recrystallised from chloroform–methanol to form yellow needles, mp 185—187° (decomp.). It gave a brown color with ferric chloride and a red color with magnesium–hydrochloric acid. λ_{max}^{EtOH} nm (log ε): 256 inf. (4.07), 271 (4.15), 295 sh (3.86), 304 sh (3.87), 340 inf. (4.06), 369 (4.13). $\lambda_{max}^{EtOH+AcONa}$ nm: 256 inf 273, 292 sh, 301 sh, 341 inf, 373. $\lambda_{max}^{EtOH+NaOEt}$ nm: 285, 325, 427. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 234, 246 inf, 274, 301 sh, 360, 433. ν_{max}^{KBr} cm^{-1}: 3350, 2900, 1652, 1621, 1565, 1490, 1450, 1375. $\delta_{ppm}^{d_6\text{-DMSO}}$: 1.67 (3H, s, CH_3 of γ,γ-dimethylallyl), 1.77 (3H, s, CH_3 of γ,γ-dimethylallyl), 3.24 (2H, d, 8, $-CH_2-$ of γ,γ-dimethylallyl), 5.18 (1H, t, 8, –CH= of γ,γ-dimethylallyl), 6.45 (1H, s, H–8), 6.89 (2H, d, 8, H–3′ and H–5′), 7.98 (2H, d, 8, H-2′ and H-6′), 10.0 (3H, b.s, OH-3, OH–7 and OH–4′, exchangeable with D_2O), 12.59 (1H, s, OH–5, exchangeable with D_2O). Mass Spectrum *m/e*: 354 (M^+). *Anal.* Calcd. for $C_{20}H_{18}O_6$: C, 65.27; H, 5.74. Found: C, 65.09; H, 5.88.

Licoflavonol Tetraacetate (III)——Licoflavonol (12 mg) was dissolved in dry pyridine (0.7 ml) and the solution was treated with acetic anhydride (0.8 ml) and left overnight at room temperature. The solution was then poured into ice-water, and insoluble material was collected and recrystallised from ethanol to give a tetraacetate as colorless needles (13 mg), mp 177—178°. λ_{max}^{EtOH} nm (log ε): 256 (4.38), 303 (4.31). $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1773, 1647, 1620, 1506, 1454, 1373. $\delta_{ppm}^{CDCl_3}$: 1.69 (3H, s, CH_3 of γ,γ-dimethylallyl), 1.75 (3H, s, CH_3 of γ,γ-dimethylallyl), 2.31 (3H, s, OAc), 2.33 (6H, s, 2×OAc), 2.45 (3H, s, OAc), 3.27 (2H, d, 8, $-CH_2-$ of γ,γ-dimethylallyl), 4.99 (1H, t, 8, –CH= of γ,γ-dimethylallyl), 7.20 (2H, d, 8, H–3′ and H–5′), 7.25 (1H, s, H–8), 7.79 (2H, d, 8, H–2′ and H–6′). *Anal.* Calcd. for $C_{28}H_{26}O_{10}$: C, 64.36; H, 5.02. Found: C, 64.07; H, 4.95.

Glycyrol (=C-13, VI)——Glycyrol was recrystallised from acetone to form colorless needles, mp 247—252° (decomp.). λ_{max}^{EtOH} nm: 228, 247, 256 inf, 349, 358 inf. ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1715, 1612, 1595, 1516, 1446. $\delta_{ppm}^{d_6\text{-DMSO}}$: 1.62 (3H, s, CH_3 of γ,γ-dimethylallyl), 1.73 (3H, s, CH_3 of γ,γ-dimethylallyl), 3.24 (2H, d, 8, $-CH_2-$ of γ,γ-dimethylallyl), 3.86 (3H, s, OMe), 5.15 (1H, t, 8, –CH= of γ,γ-dimethylallyl), 6.73 (1H, s, H–8), 6.91 (1H, q, 2 and 8, H–5′), 7.11 (1H, d, 2, H–3′), 7.67 (1H, d, 8, H–6′), 10.0 (2H, b.s, OH–5 and OH–4′, exchangeable with D_2O). Mass Spectrum *m/e*: 366 (M^+).

Licoricone (=C-18, VII)——Licoricone was recrystallised from acetone to form colorless needles, mp 255—257° (decomp.). λ_{max}^{EtOH} nm: 238, 248, 284, 302. ν_{max}^{KBr} cm^{-1}: 3100, 2900, 1625, 1608, 1581, 1505. $\delta_{ppm}^{d_6\text{-DMSO}}$: 1.66 (3H, s, CH_3 of γ,γ-dimethylallyl), 1.72 (3H, s, CH_3 of γ,γ-dimethylallyl), 3.18 (2H, d, 8, $-CH_2-$ of γ,γ-

6) T. Takemoto, K. Daigo, and Y. Tokuoka, *Yakugaku Zasshi*, **95**, 312 (1975).

dimethylallyl), 3.40 (3H, s, OMe), 3.87 (3H, s, OMe), 5.10 (1H, t, 8, –CH= of γ,γ-dimethylallyl), 6.30 (1H, s, H–5′), 6.87—7.0 (2H, H–6 and H–8), 7.88 (1H, d, 8, H–5), 7.92 (1H, s, H–2), 9.04 (1H, b.s, OH, exchangeable with D_2O), 10.7 (1H, b.s, OH, exchangeable with D_2O). Mass Spectrum *m/e*: 382 (M^+).

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